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Validity of Frozen section in the Diagnosis of Breast lumps

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Abstract: To study validity of frozen section in the diagnosis of breast lumps And To compare the results of frozen section with histopathology and to determine accuracy of frozen section.

1. Introduction:

Biopsies are used most often to determine whether cancer cells are present, although certain infections and other diseases can be diagnosed as well. A specific type of biopsy procedure called the frozen section was developed in order to make a rapid diagnosis of a mass during surgery.Welch was the first to make clinical use of the frozen section technique. In 1891, he performed frozen section microscopy on tissue which Halsted had removed during breast surgery1. Wilson developed intraoperative pathological diagnosis with frozen section technique in 1905 2. Following introduction of cryostat in 1960, frozen section examination was established as a highly reliable procedure for the rapid histological evaluation of tissue specimens during surgery, since then the method was recommended and criticized too 4.

Breast carcinoma is the second most common malignant tumor among rural Indian women after carcinoma cervix whereas in urban Indian women, breast carcinoma overcomes the incidence of carcinoma cervix 4. Clinical history and physical examination remain the most important means of detecting breast carcinoma. Although other methods have been developed to reach a preoperative diagnosis such as imprint and smear cytology, fine needle aspiration and intraoperative cytology, frozen section still plays an important role in aiding the surgeon to choose the best therapeutic approach intraoperatively.Technical skill and diagnostic expertise are essential for frozen diagnosis. Because the diagnoses made by the pathologist from frozen section have consequences for the treatment, high degree of accuracy is mandatory and quality control is important. After the introduction of frozen section facility in the Department of pathology in our institution, the need to validate the accuracy of intraoperative diagnosis by frozen section was felt by all in dealing with clinically suspicious breast lumps where preoperative FNAC was inconclusive. Hence this study to validate the intraoperative diagnosis of breast lumps by

frozen section was undertaken. The results of frozen section were compared with histopathology to determine accuracy of frozen section. Frozen sections have few major limitations such as, occurrence of potential sampling error because of the need for rapid diagnosis during a surgical procedure, only a few sections may be examined during an acceptable time interval.

2. Material and methods

All surgically operated breast lesions submitted for intraoperative diagnosis to the histopathology department, MGM Medical College and Hospital, Aurangabad during a period of October 2014 to October 2016 are included in this prospective study.Sample Size: 100 patients Both male and female patients of any age having lump which is clinically suspicious of malignancy were included in the study. It was made mandatory to give call to the resident/pathologist on call 4 hours before starting the surgery so as to establish required temperature of cryostat. Patients who were subjected to incisional biopsy or lumpectomy depending on size of lump and specimen were analyzed by frozen section as well as by histopathology section. The results of frozen section were compared with final diagnosis reached by histopathology.
The gross specimens of the tumours were examined, painted and cut into thin slices. Tissue bits were sampled from abnormal and suspected areas, or from the firm lesions, frozen immediately to -25C0, sectioned at 4-5 microns using YORCO Cryostat and stained with rapid haematoxylin and eosin..The resident doctors and technical staff were delegated responsibility according to their experience. Microscopic findings were reported to the surgeon verbally in the operating room and were immediately recorded in a book. After completion, remaining unfrozen tissue was fixed in 10% neutral buffered formalin solution, processed and embedded in paraffin. Permanent histological sections were obtained , stained with H & E and compared with frozen section.
A frozen section diagnosis of infiltrating neoplasm was reported only if unequivocal evidence was available, whereas in situ carcinoma was assumed as a temporary diagnosis needing confirmation. Whenever any doubt existed, the diagnosis was deferred to paraffin sections and the deferrals were categorized as being due to technical imperfection, sampling error, the focal nature of the lesion or morphological misinterpretation of the appearances of frozen section. The results and causes of concordant, discordant and deferral cases were obtained on a predesigned proforma respectively. A minimum of 100 cases with following inclusion criteria were selected for study.Both male and female patients clinically suspicious of malignancy are included in the study.

3. Material and Equipment Required:

Microscopic glass slides, glass marking pensil, Labels Fixatives (Freshly prepared acetic acid formal saline for frozen and ethanol for histopathology) Staining equipment (H& E stain)Completed requisition form with all clinical details.
Microscope.
OCT Medium.
Camel hair paint brush 0 number.

4. Procedure for Frozen Section:

1. Obtain frozen tissue. It is imperative that the tissue be frozen as quickly as possible in order to avoid ice crystal formation resulting in artifact and poor morphological preservation.

2. Make sure the cryostat is at proper operating temperature -25° C to -30° C. Place a small amount of OCT or other suitable frozen section embedding medium on a cryostat object disk

(make sure the disk is at room temp. before mounting the specimen).

3. Position the frozen specimen in the center of the object disk and place the disk on the cryobar in the cryostat to begin the quick freeze process.

4. Before the disk is frozen solid, add enough OCT to cover the top the specimen.

5. Place the object disk in the microtome object disk holder and tighten the set screw or clamp.

6. Make sure that there is enough clearance between the block and the microtome knife.

7. Move the block toward the knife edge. Make sure the ratchet is disengaged from the micrometer gear. Turn the flywheel with the

right hand and begin turning the gross adjust wheel (on the down stoke) slowly with the left hand. Face off enough OCT until a full section of the specimen is visible.

8. Engage the ratchet on the micrometer gear, cut and discard the first two or three sections.

9. Have the proper fixative (Freshly prepared acetic acid formalin for rapid H&E) and slides ready. Turn the flywheel with the right hand. As the block comes in contact with the knife edge the section will move down the blade and begin to curl. Hold the section down with as little force as possible and guide in along the blade using a camel hair paint brush in the left hand. Continue the cut until a full specimen section has been obtained, but stop before passing through the remaining OCT. One edge of the section is held flat with the paint bush and the other with the knife edge.

10. Pick up the slide with the right hand and turn it so that the top side is facing toward the knife blade.

11. Carefully lower the slide onto the blade, keeping the slide parallel to the section. As the tissue comes into contact with the slide the OCT and tissue will melt causing the tissue to adhere to the slide.

5. Procedure of Staining Frozen Section Staining by Rapid H & E

Keep all stains and solutions fresh and well maintained Dip slide in reagents in this order for H&E staining:
-After obtaining frozen section, immediately fix in freshly prepared acetic acid formalin (even 15 seconds of delay can cause significant artifact).
-Formal Alcohol, Formalin or 95% alcohol: --45-60 seconds.
-Water: 5-7 seconds.
- Hematoxylin: 60 seconds.
- 0.2 % aqueous ammonia (Bluing): 15-20 seconds
- Eosin: 20-60 seconds.
- 95% alcohol: 10 seconds.
- 100% alcohol: 10 seconds.
- Xylene : 10 seconds.
- Then add mounting media for cover slipping.Diagnostic validity tests was performed to assess the efficacy of frozen section in breast lumps. Categorical data were subjected to Chi-square test.

6. Results:

The present prospective study comprises of 100 cases with breast lumps referred by surgery department, which were subjected to frozen section intraoperatively which were processed at pathology department by investigator himself of MGM Medical College , Aurangabad during period of October 2014 to October 2016.

Number of breast lump cases were 100, out of these maximum cases were found in 5th decade i.e. 30 cases (30%) and minimum in 2nd decade i.e. 1 case (1%). In the present study , breast lumps were located maximum in upper outer quadrant i.e.46 cases (46%) and minimum in upper outer and upper inner i.e. 1 case (1%).

Histopathological distribution of benign and malignant breast diseases:

Out of 100 cases , 61 cases (61%) were diagnosed as benign however 39 cases (39%) were diagnosed malignant on histopathology.

Table 4: Histopathological distribution of Benign and Malignant Breast Diseases

Breast diseases	Cases	
	No.	%
Benign	61	61%
Malignant	39	39%

Distribution of benign breast diseases on histopathology diagnosis

Out of 61 cases diagnosed as benign on histopathology, 32 cases (52%) were reported as fibroadenoma , 16 cases (26%) as fibrocystic disease, 07 cases (12%) as epithelial hyperplasia, 02 cases (3%) as benign phyllodes and 4 cases (07%) as granulomatous mastitis

Table 5: Distribution of Benign Breast Diseases on Histopathology diagnosis

Benign Disease	No. of cases	Percentage
Fibroadenoma	32	52%
Fibrocystic disease	16	26%
Epithelial hyperplasia	07	12%
Benign phyllodes	02	3%
Granulomatous mastitis	04	7%
Total	**61**	**100%**

Distribution of malignant breast diseases on histopathology

Out of 39 malignant cases diagnosed on histopathology, 26 cases were infiltrating duct carcinoma, followed by 4 cases of ductal carcinoma in-situ , 3 cases of malignant phyllodes followed by 2 cases each of medullary and mucinous carcinoma.

Table 6: Distribution of Malignant Breast Disease on Histopathology diagnosis

Malignant Disease	No. of cases	Percentage
Ductal carcinoma in-situ	04	10.25
Infiltrating duct carcinoma	26	66.66
Infiltrating lobular carcinoma	02	5.12
Medullary carcinoma	02	5.12
Mucinous carcinoma	02	5.12
Malignant phyllodes	03	4.34
Total	**39**	**100%**

Table 7: Correlation of Frozen section with Histopathological diagnosis

FS Diagnosis	Total No.	HP Diagnosis		False positive	False Negative	Accuracy %
		Benign	Malignant			
Benign	62	61	01	0	1	98.37%
Malignant	38	00	38	0	0	
Total	100	61	39	0	1	

In the present study, out of 100 cases 62 cases were diagnosed as benign and 38 cases as malignant on frozen section . However on histopathology 61 cases were diagnosed as benign and 39 cases as malignant. One of the case diagnosed as ductal carcinoma in-situ on histopathology was reported as epithelial hyperplasia on frozen section.

7. Discussion:

Breast carcinoma is one of the commonest cancer among females in India preceded only by cervical cancer 7. Early screening and diagnosis of breast lesions can aid in prevention as well as accurate management of the patients thus alleviating discomfort and anxiety in the process . In present.

study, benign breast lesions were diagnosed in 61 cases (61%) and malignant breast lesion in 39 cases (39%) by histopathology. Benign breast diseases are 4-5 times more common than malignant breast diseases [74,78]. The maximum number of benign breast lesions was found in the age group of 31-40 years, 23 cases (37.7%) followed by 41-50 years,18 cases (29.5%). Malignant breast lesions were more common in the age group of 41-50 years and 51-60 years, 12 cases (30.76%) each followed by 61-70 years, 7 cases (17.94%). Age is an important risk factor for breast diseases, especially malignant. The breast cancer risk

increases as the age advances. In the present study, mean age of cases was 45.2 13.32 years. Other studies also reported similar findings 9.

The Upper outer quadrant was the most commonly involved segment (44%) in the present study. Central (21%), upper inner (13%) and lower outer (10%) were the other common locations. On comparing this study with that done by Oluwole and Freeman [82], the result was, upper outer quadrant was the most commonly involved part of the breast. The explanation given is that, as the maximum breast mass is situated in upper outer quadrant, breast lesions are more commonly found in this quadrant.

The importance and the contribution of frozen-section diagnosis (FSD) during surgery is now a well-established procedure for the rapid diagnosis of surgical specimens. Until 1960, most pathology laboratories did not use the FSD procedure as a routine method because of the various technical disadvantages. After the introduction of the cryostat, the technique was greatly improved and during the last 20 years this procedure has become established as a routine method for all kinds of biopsy.

The major indication for the use of this method is to allow the surgeon to make an immediate therapeutic decision, thus sparing the patient possible reoperation and reducing the length and cost of hospital treatment. Since the diagnosis made by the pathologist from frozen section may have serious consequences for the treatment of the patient, a high degree of accuracy is mandatory and quality control is important The false negative case in the present study which on frozen section was reported as atypical ductal hyperplasia (ADH) was diagnosed histopathologically as ductal carcinoma in-situ .Most significant factor contributing this false positive diagnosis was freezing artifact produced by frozen section because which it was misinterpretated as ductal hyperplasia.

8. Conclusion:

Few diagnostic techniques can have as immediate and serious consequences in the treatment of a patient as the frozen section performed during a surgical operation. If high standards of accuracy are not established and met, the pathologist cannot reliably fulfill an often vital role in diagnosis and subsequent therapy. Freezing artifacts could pose problem for the pathologist in reporting frozen section and could significantly delay turnaround time. Thus it is necessary to maintain high standards of accuracy and timely monitoring of turnaround time.Limited sampling offered by frozen section could pose diagnostic difficulty for pathologist in doubtful cases. So such cases should be deferred for histopathological diagnosis.In present study the diagnostic sensitivity, specificity and efficiency of the frozen section method were high. In the hands of trained and experienced pathologists, FS is one of the most accurate diagnostic procedures currently available, and its reliability and high degree of accuracy in palpable breast lesions is well established.

It is proven that FSD of various breast lesions is a highly reliable procedure in spite of the difficulties that the pathologist faces with the frequent borderline or premalignant lesions. This again emphasize the great responsibility of the pathologist in FSD.To conclude, frozen section is an accurate and useful test in the intraoperative evaluation of patients with suspected breast carcinomas. Its results can be used to guide the type and extent of surgery, especially in institutions with experienced pathologists. The accuracy of frozen section is very high for benign and malignant tumors. Every effort should be made to establish this procedure in institutions that treat large numbers of patients with breast carcinomas. However histopathology is still a gold standard.

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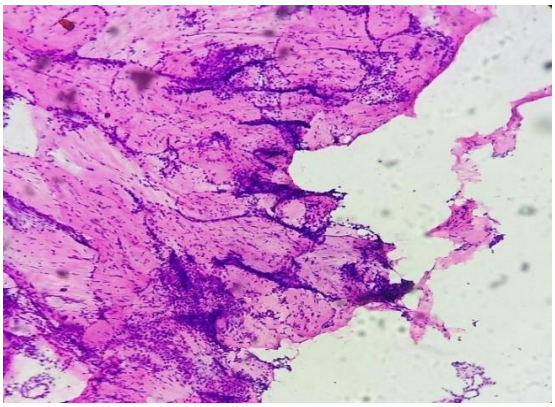
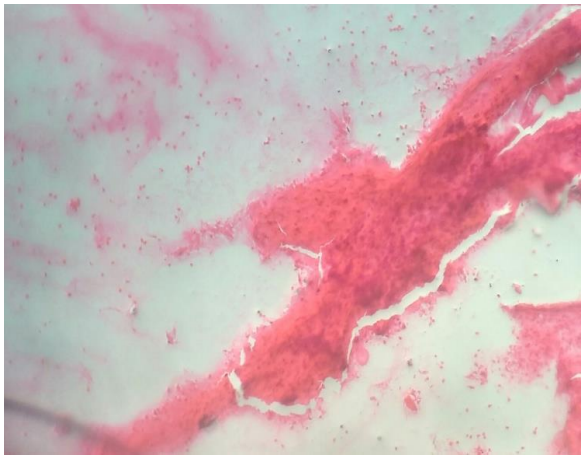
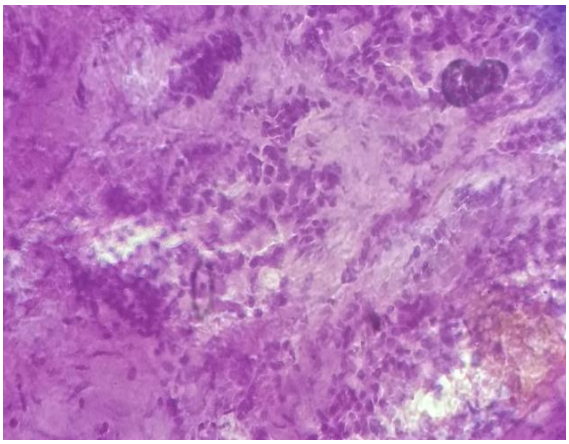


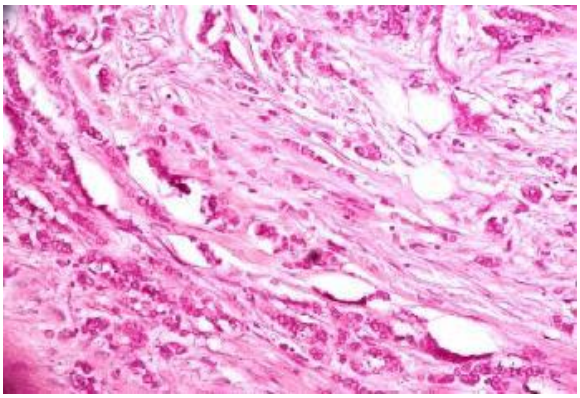
Photomicrograph of frozen section of fibroadenoma revealing benign stromal elements with few compressed ducts (Rapid H & E ,10X)



Photomicrographs of frozen section of benign phyllodes showing benign stromal proliferation (Rapid H & E, 10X)



Photomicrograph of frozen section of infiltrating duct carcinoma showing round to polyhedral cells with marked atypia distributed throughout the stroma. (Rapid H & E, 40X)



Photomicrograph of frozen section of infiltrating lobular carcinoma showing Indian file arrangement (Rapid H & E, 10X)

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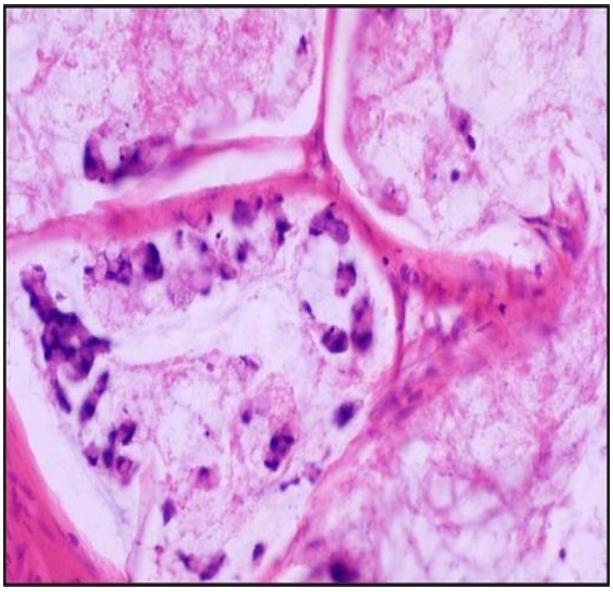

Photomicrograph of frozen section of mucinous carcinoma of breast showing round to polyhedral cells floating in pools of mucin with abundant collagenous stroma.(Rapid H & E, 10X)

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